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(54) Title: PREVENTION, THERAPY AND PROGNOSIS/MONITORING IN SEPSIS AND SEPTIC SHOCK

(57) Abstract: The invention relates to the use of a peptide which binds to lipopolysaccharide (LPS) or lipoteichoic acid (LTA), for manufacturing a pharmaceutical composition for treating sepsis or septic shock, wherein the peptide comprises the amino acid sequence of apolipoprotein CI (apoCI) or a part thereof that comprises at least the amino acids of the C-terminal helix of apoCI. The use of human apoCI is preferred. The peptide can be administered clinically to patients who have sepsis or threaten to develop sepsis. Measurement of the apoCI content in blood can be utilized for determining the severity and prognosis of the course of the septic condition or for monitoring an anti-sepsis treatment.

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Title: Prevention, therapy and prognosis/monitoring in
sepsis and septic shock

FIELD OF THE INVENTION

The invention is in the field of medicine, more particularly the field of prognosis, prevention and therapy
5 of sepsis and septic shock. The invention provides means for therapeutic prevention and treatment of sepsis and septic shock, as well as means to determine the severity of a septic condition and to make a prognosis for the further course, and means for septic monitoring of the course of a
10 treatment of sepsis and septic shock.

BACKGROUND OF THE INVENTION

Sepsis and septic shock.

15 A normal bacterial inflammation is a highly regulated process whereby the defence system clears up bacteria. In humans with a weakened defence, the bacterium gets an opportunity to proliferate (virtually uninhibitedly) and to break through to the blood stream.
20 The presence of bacteria in the blood stream is called sepsis. Sepsis leads to the production of proinflammatory mediators by white blood cells (monocytes and macrophages) in the blood. This reaction is crucial for a fast and efficient elimination of bacteria. However, if the
25 inflammatory reaction persists too long, for instance due to disease-related immune suppression, this may eventually lead to severe fever, spontaneous blood clotting and damage of tissues due to oxygen deficiency and due to bactericidal products. In serious cases, organs (specifically kidney,

heart and liver) may stop functioning and the patient may get into so-called septic shock, from which he may subsequently die.

5 ***Incidence.***

In the United States alone, more than 500,000 patients a year develop septic shock, with an estimated incidence increase of about 1.5% a year, and the costs amount to \$16.7 billion a year (Angus, 2001). Septic shock results in death in 20-80% of cases (depending on the definition used). The increasing use of invasive surgical techniques, chemotherapy and the application of immunosuppression in patients with organ transplants or inflammatory diseases constitute causes of an increasing number of cases of sepsis. The longer life expectancy of elderly people and patients having metabolic, neoplastic and immunodeficiency defects owing to improved medical care, also leads to populations at increased risk of bacterial infection.

20

Lipopolysaccharide and lipoteichoic acid.

Sepsis and septic shock are caused chiefly by Gram-negative bacteria (for instance *E. coli* and *K. pneumoniae*) and (to a lesser extent) Gram-positive bacteria (*S. aureus* and *S. epidermidis*).

25

Lipopolysaccharide (LPS) is the most important constituent of the outer membrane of Gram-negative bacteria. LPS is the most reactive component of Gram-negative bacteria. LPS activates mononuclear cells (monocytes and macrophages), after which these cells produce proinflammatory mediators such as cytokines (TNF α ,

30

IL-1 β , IL-6) and oxygen radicals. This response is necessary for a fast and effective dealing with the bacterial infection, and plays a crucial role in the clearing up of the bacteria (Heumann, 2002). However, in case of an inadequate response, allowing the bacteria to continue to proliferate uninhibitedly, these mediators are responsible for the metabolic changes that lead to pathologic conditions and eventually to death, as demonstrated in rabbits, monkeys and mice.

10 The equivalent of LPS in Gram-positive bacteria is lipoteichoic acid (LTA), which possesses a structure that exhibits strong homology to that of LPS.

Current clinical and experimental therapies.

15 The complexity of sepsis and the immunological defense render the development of pharmacological interventions more difficult. The standard treatment often consists in the administration of fluid and blood pressure raisers (so-called vasopressors) to normalize the blood pressure and the oxygen supply of organs, and antibiotics to inhibit the proliferation of bacteria (Rackow, 1991; Cohen, 1991; Wheeler, 1999), but this treatment is not sufficient to prevent the high mortality.

20 Most applied experimental strategies for treating sepsis have been aimed at inhibiting the proinflammatory response, for instance through capturing LPS, blocking the cytokine action or overall immune suppression.

25 However, various clinical studies have shown that LPS-neutralizing antisera are not effective in septic shock (Cohen, 1999). Likewise, experimental therapies with antibodies to *inter alia* TNF α (Fisher, 1996; Clark, 1998)

and the IL-1 receptor (Fisher, 1994; Opal, 1997) proved not to be effective.

By means of a meta-analysis, it has been demonstrated that overall immune suppression increases the mortality resulting from sepsis (Cronin, 1995). A recent study in humans demonstrates that a polymorphism in the LPS receptor Toll-like receptor 4 (TLR4), which leads to a reduced inflammatory response against LPS, is associated with an increased sensitivity to severe bacterial infection (Kiechl, 2002). Furthermore, it is known that mice, which are deficient for the proinflammatory cytokine macrophage migration inhibitory factor (MIF), are less well capable of clearing up bacteria such as *Leishmania major* (Satoskar, 2001) and *Salmonella typhimurium* (Koebernick, 2002).

Many suggestions have been made for treating sepsis or septic shock, all of which have in common that an LPS-binding peptide is administered to sepsis patients. The administration of these LPS binding peptides is typically aimed at the suppression of the LPS-induced activation of the immune system. The peptides to be administered can, for instance, be derived from LBP (LPS Binding Protein; see WO 95/25117), apoA and apoE (WO 98/07751), apoA1 (WO 95/25786; WO 99/16458; WO 99/16459; WO 99/16408; WO 99/16409), CAP18 (Larrick, 1994), CAP37 (US 6107460; US 5650392; US 5627262; US 5607916), CD14 (WO 96/20956), prophenin (WO 95/34289; WO 95/26747), polyphemusin (WO 02/00687) and LALF protein (US 5747455). To date, however, all of these suggestions have not yielded an effective approach for sepsis and septic shock either.

Sepsis has great consequences for the lipoprotein metabolism. Patients who get to the clinic exhibit strongly

lowered cholesterol levels in the blood, which normalize after successful antibacterial therapy. Research *in vitro* and in experimental animals has demonstrated that lipoproteins can offer protection against sepsis, but the complexity of lipoproteins (including composition, endogenous character) makes their clinical application more difficult. Also, the mechanism by which lipoproteins play a protective role is still unknown. It has been reported that infusion of protein-free triglyceride-rich lipid emulsions does not afford protection (Van der Poll, 1995), which indicates that it is precisely the protein components of lipoproteins that can play a large protective role in sepsis.

15 BRIEF DESCRIPTION OF THE INVENTION

An object of the invention is to provide a prevention or effective handling (therapeutic treatment) of sepsis and septic shock, which is more efficient than the treatment methods proposed heretofore and/or avoids disadvantages thereof.

Another object of the invention is to provide means and methods by which the proinflammatory reaction as induced by the toxic components of bacteria, in particular LPS and/or LTA, in septic patients can be enhanced.

A further object of the invention is to provide a reliable determination of the severity of a septic condition and reliable prognosis of sepsis or septic shock.

A still further object of the invention is to provide a reliable evaluation of the pathologic condition

of septic patients, so that the effect of a treatment of the disease can be measured.

To that end, the invention provides the use of a peptide which binds to lipopolysaccharide (LPS) or lipoteichoic acid (LTA), for manufacturing a pharmaceutical composition for treating sepsis or septic shock, the peptide comprising the amino acid sequence of apolipoprotein CI (apoCI) or a part thereof that comprises at least the amino acids of the C-terminal helix of apoCI.

10 In particular, this concerns a peptide which comprises the amino acid sequence of human apoCI or a part thereof that comprises at least the amino acids of the C-terminal helix of human apoCI. However, apoCI of a non-human mammal, or a corresponding fragment thereof, can also
15 be used. For applications in, e.g., domestic animals, such as horse, cow, dog and cat, typically the use of an apoCI of the respective mammal will be preferred.

Most preferred according to the invention, however, is that the peptide is human apoCI or a fragment thereof
20 that comprises at least the amino acids MREWFSETFQKVKEKLLK.

Concretely, the peptide is preferably human apoCI having the amino acid sequence TPDVSSALDKLKEFGNTLEDKARELISRIKQSELSAKMREWFSETFQKVKEKLLKIDS. However, it is also possible to use a fragment, for instance the peptide SAKMREWFSETFQKVKEKLLKIDS or
25 the peptide MREWFSETFQKVKEKLLK.

In particular, this concerns the use of a peptide which binds to lipopolysaccharide (LPS), as defined herein, whereby a pharmaceutical composition is manufactured for preventing or treating a sepsis or septic shock caused by
30 Gram-negative bacteria in mammals, in particular humans or a domestic animal, such as horse, cow, dog and cat.

However, the invention also comprises the use of a peptide that binds to lipoteichoic acid (LTA), as defined herein, whereby a pharmaceutical composition is manufactured for preventing or treating a sepsis or septic shock caused by Gram-positive bacteria in mammals, in particular humans or a domestic animal, such as horse, cow, dog and cat.

Also, according to the invention, there is provided a pharmaceutical composition for preventing or treating sepsis or septic shock, which composition comprises a peptide as defined herein, as well as a pharmaceutically acceptable carrier.

Further, the invention provides a method for preventively treating a mammal, in particular human individual, which, for instance as a result of a surgical intervention or a weakened or deficient immune system, is at increased risk of developing sepsis, wherein an active amount of a peptide as defined herein is administered to the mammal.

Likewise, the invention provides a method for treating a mammal, in particular human individual, which suffers from sepsis or septic shock, wherein an active amount of a peptide as defined herein is administered to the mammal.

Also, the invention provides a method for determining the severity of a septic condition and making a prognosis for the further course of the sepsis or septic shock in a mammal, in particular human individual, which is suffering from sepsis or septic shock, wherein the apoCI content in a blood sample of the mammal is determined.

Likewise, the invention provides a method for monitoring a treatment of sepsis or septic shock in a mammal, in particular human individual, which is being treated for sepsis or septic shock, wherein the apoCI
5 content in a blood sample of the mammal is determined.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the relation between the apoCI level
10 in the blood plasma of sepsis patients and the lapse of time after admission to the clinic. The figure demonstrates the predictive value of the apoCI level for the chance of survival of sepsis.

Figure 2 shows the effect of an electrophoretic
15 field on the migration of apoCI and/or LPS through an agarose matrix. The figure demonstrates a physical interaction between apoCI and LPS, which leads to comminution of LPS aggregates and also to reduced recognition of apoCI by a specific antibody.

Figure 3 shows the relation between the molar
20 LPS:apoCI ratio and the inhibition of the stain reaction during ELISA by which the concentration of apoCI is determined. The figure shows a strong interaction between apoCI and LPS, which can prevent the binding of a specific
25 antibody to apoCI.

Figure 4 shows the effect of apoCI and apoCIII on the time-dependent deaggregation of LPS. The figure shows a dose-dependent monomerization of LPS micelles by apoCI, while apoCIII is hardly effective.

Figure 5 shows the effect of the apoCI concentration
30 in murine plasma on the time-dependent monomerization of

LPS. The figure shows that the absence of apoCI strongly inhibits the monomerization of LPS, while expression of human apoCI increases the monomerization of LPS dose-dependently.

5 **Figure 6** shows the effect of apoCI and bovine serum albumin on the behavior of radioactively labeled LPS after intravenous injection in mice. The figure shows that apoCI is capable of strongly reducing the uptake of LPS by the liver, while the residence time of LPS in the blood
10 increases considerably through the apoCI-induced binding of LPS to HDL. Bovine serum albumin, by contrast, proved not effective.

Figure 7 shows the effect of apoCI on the LPS-induced proinflammatory response. The figure shows that the
15 interaction of LPS with apoCI strongly increases the inflammatory response (i.e. TNF α induction).

Figure 8 shows the correlation between the apoCI levels in the plasma of patients who develop endotoxemia during a heart operation with cardiopulmonary bypass, with
20 pre- and post-operative TNF α levels. The figure shows that there is a strongly significant positive relation between the apoCI level and the TNF α level in patients who develop endotoxemia, but not in patients who do not develop endotoxemia. Such a relation could not be demonstrated for
25 apoCIII or apoE.

DETAILED DESCRIPTION OF THE INVENTION

Role of apoCI in sepsis.

 We have found, as Example 1 and Figure 1 show, that
30 the level (the concentration) of apolipoprotein CI (apoCI) in septic blood is strongly lowered, which does not hold,

or holds to a much lesser extent, for the (numerous) other apolipoproteins. This lowered apoCI content in septic blood is confirmed by the literature (Barlage, 2001), although the authors have not discussed a possible role of apoCI in sepsis.

ApoCI is the smallest apolipoprotein (6.6 kDa, 57 amino acids) which is chiefly synthesized by the liver and circulates in the blood in a fairly high concentration (~6 mg/dL), both in free, unbound form and as a constituent of lipoproteins (chylomicrons, VLDL, HDL) (Jong, 1999). The amino acid sequence of apoCI is known (see Table 1). To date, no mutations have become known that lead to different variations ('polymorphisms') of apoCI. Using transgenic mice that overexpress the human apoCI, we have demonstrated that apoCI plays a role in the lipid metabolism, since apoCI overexpression leads to increased lipid levels in the blood (Jong, 1996; Jong, 1998), but a role, if any, of apoCI in sepsis was not yet known.

We have developed the hypothesis that the apoCI depletion associated with sepsis is a direct consequence of bacterial infection. The low apoCI concentration might be the consequence of binding of apoCI to bacterial products, specifically LPS, which may possibly lead to accelerated clearance of apoCI from the blood.

Via an extensive literature study, it was found that LPS-binding proteins have common characteristics, with the presence of a combination of positively charged and hydrophobic amino acids being essential. Two of these proteins are the limulus anti-LPS factor (LALF) (Hoess, 1993), a factor in the 'LAL assay' routinely used to

measure the LPS activity, and CAP18 (Larrick, 1994), which is synthesized by polymorphonuclear cells.

In fact, apoCI contains a large number of positively charged lysines (one in each six amino acids), alternated
 5 with hydrophobic amino acids. To our surprise, human apoCI contains a peptide sequence in its C-terminal portion (**KVKEKLK**) which is virtually identical to the LPS-binding sequences of LALF (**KWKYK***GK*) and CAP18 (**KI***KEKLK*), where symbols in bold-type and italics respectively represent
 10 positively charged and hydrophobic amino acids. We have subsequently demonstrated that apoCI, both in pure form and as a constituent of plasma in fact binds strongly to LPS (see Example 2 and Figures 2, 3, 4 and 5).

We have compared the amino acid sequences of apoCI,
 15 such as occur in man (SwissProt P02654), baboon (SwissProt P34929), dog (SwissProt P56595), rat (SwissProt P19939) and mouse (SwissProt P34928), as can be found under the numbers indicated (<http://www.expasy.ch/sprot/>). See Table 1.

20 **Table 1. Primary amino acid sequences of apoCI.**

Human apoCI (1-57)

TPDVSSALDKLKEFGNTLEDKARELISRIKQSELSAKMREWFSETFQ**KVKEKLK**IDS

Baboon apoCI (1-57)

25 APDVSSALDKLKEFGNTLEDKAWVINRIKQSEFFPAKTRDWFSETFR**KVKEKLK**INS

Dog apoCI (1-62)

AGEISSTFERIPDKLKEFGNTLEDKARAAIESIKKSDIPAKTRNWFSEAF**KKVKEHLK**TAFS

Rat apoCI (1-62)

APDFSSAMESLPDKLKEFGNTLEDKARAAIEHIKQKEIMIKTRNWFSETLN**KMKEKLK**TTFA

30 **Mouse apoCI (1-62)**

APDLSGTLESIPDKLKEFGNTLEDKARAAIEHIKQKEILTKTRAWFSEAF**GKVKEKLK**TTFS

The consensus LPS-binding sequence underlined in Table 1 as found in human apoCI (KVKEKLLK) has been found to occur virtually unmodified in the animal variants. Human apoCI forms two amphipathic helices of class 2 (amino acid sequences 7-29 and 38-52), which facilitate the binding of apoCI with lipoproteins. On the basis of structural homology, it is assumed that animal apoCI takes a comparable structure.

10 ***Working mechanism of apoCI.***

On the basis of the disappointing results of the earlier discussed, failing experimental antiseptic therapies, we have furthermore developed the hypothesis that therapeutic reduction of the proinflammatory response is not only ineffective but even undesirable, because in this way the causative bacterial proliferation is not inhibited. Although the cause of sepsis is very heterogeneous and the time of intervention may be determinative of the success of the treatment, we deem it likely that specifically enhancement of the inflammatory response in an early stage of sepsis increases the antibacterial effectiveness and hence raises the chance of survival.

While proteins, such as CAP18, LALF, CAP37, prophenin and polyphemusin, which do not form part of lipoproteins, are also capable of binding LPS, they do not, in contrast with apoCI, occur in a high concentration in the blood. Further, these proteins lead to suppression of the LPS-related inflammation cascade, which according to our hypothesis is unfavorable for the course of the

disease. The bacterial infection will, as a result, aggravate rather than improve.

Surprisingly, we have now established that the binding of LPS to human apoCI leads to a considerably longer residence time of LPS in the blood (see Example 4 and Figure 6), owing to its binding to plasma lipoproteins, facilitated by the amphiphilic structure of apoCI. Also, we have established that administration of LPS with apoCI to mice leads to a strongly increased proinflammatory response compared with mice that were administered LPS alone, which appears from a fourfold increased TNF α production (see Example 5 and Figure 7). The finding that apoCI enters into a direction interaction with LPS is new and points to a direct role of apoCI in the endogenous control of bacterial sepsis.

Because apoCI is a constituent of lipoproteins (chylomicrons, VLDL and HDL) and is consequently cleared slowly from the blood, we therefore suppose that the binding of LPS to apoCI extends the residence time of LPS in the blood, so that the activation of white blood cells is additionally stimulated in a natural way. As a result, the production of proinflammatory cytokines (TNF α , IL-1 β , and IL-6) will increase and consequently the antibacterial inflammation cascade will work extra strongly. This mechanism can also partly explain why lipoproteins play a protective role in sepsis. Accordingly, the essence of the present invention is that through administration of apoCI, or an adequate fragment thereof, as defined herein, a timely, extra forceful inflammation cascade is obtained which enables early neutralization of both LPS and the LPS-

producing bacteria, so that the stage of full-blown sepsis or septic shock will not be reached.

Clinical applicability.

5 The small size of apoCI enables obtainability of the protein on a large scale through so-called solid-phase peptide synthesis. This holds to a greater extent for smaller peptides (fragments of apoCI), which bind LPS and stimulate LPS-induced activation of white blood cells.

10 Since apoCI is an endogenous plasma protein, it will be tolerated well and not generate any (additional) immune reactions. (For that reason, use of human apoCI will be preferred in humans, while in animals, preferably the corresponding animal apoCI will be used).

15 It is noted that according to the invention, apoCI, or a suitable fragment thereof, is used as the active substance to control or prevent sepsis or septic shock. According to the invention, the apoCI is not used as carrier for another substance. According to the invention,

20 the apoCI, or fragment thereof, is preferably used as free peptide, not bound in complexes with lipids.

Measurement of apoCI content for prognosis or monitoring.

 As is demonstrated in Example 1 and Figure 1,

25 patients having severe sepsis exhibit a strongly lowered apoCI level. In the example given (17 patients admitted to the clinic with severe sepsis) the apoCI level was on average 1.6 mg/dL. The apoCI levels in patients who eventually survived the sepsis (47%: 8/17) exhibited a

30 progressive rise after 3 days and normalized after about 4 weeks. By contrast, the apoCI levels in the patients who eventually died from the sepsis within 4 weeks (53%: 9/17), did not exhibit any rise. These data show that monitoring of the apoCI concentration in blood plasma has a prognostic

35 value for the chance of survival of the septic patient (see

also Figure 1), and can be used for monitoring the effect of antiseptic therapies.

A. The peptides to be used according to the invention.

5 As set out above, the invention comprises the application of apoCI and peptides derived therefrom which bind to LPS for the therapeutic treatment of Gram-negative bacterial sepsis. However, since apoCI and peptides derived therefrom also bind to lipoteichoic acid (LTA), the toxic
10 equivalent of LPS in Gram-positive bacteria, the invention comprises the use of apoCI in bacterial infections in general, hence also in Gram-positive bacterial sepsis.

 According to a particular preferred embodiment, the peptide consists of the whole amino acid sequence of apoCI
15 (or comprises this sequence), in particular that of human apoCI, which consists of 57 amino acids according to the amino acid sequence

TPDVSSALDKLKEFGNTLEDKARELISRIKQSELSAKMREWFSETFQKVKEKLIKIDS.

 According to another preferred embodiment, the peptide
20 is, or comprises, the amino acid sequences of homologues of human apoCI such as they occur in the animal kingdom (in particular in mammals, for instance horse, cow, dog, rabbit, rat, mouse).

 According to another preferred embodiment, the
25 peptide is, or comprises, at least the C-terminal helix of apoCI. In human apoCI, the C-terminal helix is formed by the amino acids 38-52, with the amino acid sequence MREWFSETFQKVKEK. Preferably, a useful fragment of human apoCI comprises at least the amino acids MREWFSETFQKVKEKLIK.
30 Examples of suitable peptides are, for instance, the peptides MREWFSETFQKVKEKLIK and SAKMREWFSETFQKVKEKLIKIDS. These peptides comprise the C-terminal helix of apoCI and the whole putative LPS binding sequence KVKEKLIK. Instead of a fragment of human apoCI, a corresponding fragment of an
35 animal apoCI can be used.

A property important according to the invention of apoCI and the peptides defined herein, is, besides their binding to LPS, their binding to lipoproteins.

5 **B. Obtaining the peptides.**

The complete human apoCI protein (amino acids 1-57) is commercially available (BIODESIGN International, Maine, USA and INTRACEL Corporation, Maryland, USA). Also, procedures have been described to isolate human apoCI as
10 pure protein from blood (Tournier, 1984; Jackson, 1986). Finally, there is the possibility of constructing an adenoviral expression vector that codes for human or animal apoCI, so that apoCI can be produced on a large scale by astrocytoma cells in culture (Kypreos, 2001). Because apoCI
15 is a very small protein, there is also the possibility of obtaining it from the individual amino acids by means of solid phase peptide synthesis (Clark-Lewis, 1986). This technique also enables synthesization of all human apoCI analogs and other apoCI-derived peptides as described
20 herein.

C. Administration forms and routes.

Human apoCI is water-soluble and so can be administered intravenously in an aqueous solution (for
25 instance in a physiological saline solution). Single-time (bolus) injection is a possibility, as is continuous infusion.

Also, apoCI has lipid-binding properties, which further permits intravenous administration as a constituent
30 of commercially available triglyceride-rich lipid emulsions (e.g. Intralipid®, Intrafat® and Lipofundin®) which are clinically routinely used as efficient parenteral energy source. In this case, too, bolus injection and continuous infusion both are possibilities.

All this likewise holds for the peptides according to the invention.

Oral administration of peptides according to the invention and of apoCI in particular is in principle also
5 possible (Buclin, 2002).

Pharmaceutically acceptable carriers that are suitable for these different administration routes are naturally known to those skilled in the art.

10 ***D. Therapeutic dose.***

The plasma concentration of apoCI in healthy individuals is on average 6 mg/dL (Curry, 1981), while we have demonstrated that apoCI in septic patients is strongly lowered. It is expected that a plasma concentration of
15 0.05-500 mg/dL (preferably 0.5-50 mg/dL) can be therapeutically effective.

Based on an average plasma volume of 2.5 L given a body weight of 70 kg, it can be calculated that a dose of 0.02 mg/kg-200 mg/kg (preferably 0.2 mg/kg-20 mg/kg) can be
20 effective in the case of a single-time (bolus) injection.

When administered as infusion, the dose will be strongly dependent on the rate of clearance of the apoCI from the blood, so that the dosing can vary within wide limits, for instance from 0.01 to 10 mg/kg/minute.

25

E. Expected effect of the treatment.

It is expected that apoCI will bind freely circulating LPS and LTA and will keep them present in the blood longer, so that LPS and LTA will be able to induce an
30 increased proinflammatory antibacterial reaction, so that bacteria and LPS are neutralized faster and more effectively. Both preventive application of apoCI in individuals at increased risk of developing sepsis (for instance in the case of major surgery, or with immune
35 deficiency) and application of apoCI in sepsis or septic

shock will lead to the prevention of death resulting from sepsis.

F. Prognosis and monitoring of sepsis or septic shock.

- 5 The apoCI level in blood plasma can be established in any suitable manner. Preferably, the apoCI level is determined with a sandwich Enzyme-Linked ImmunoSorbent Assay (sandwich ELISA). To this end, for instance 96-wells
- 10 plates (medium-binding plates; Costar) are coated with a (10,000-fold dilution of a) polyclonal antibody to human apoCI generated in goat (goat-anti human apoCI; Academy Bio-Medical Company, Houston, USA; cat. 31A-G1b). Next, human blood plasma or serum is added (100,000-300,000-fold dilution), whereby the apoCI present therein is
- 15 quantitatively bound by this primary antibody. The apoCI is recognized by a secondary antibody (15,000-fold dilution) which is coupled to horseradish peroxidase (HRP) (HRP-goat-anti human apoCI; Academy Bio-Medical Company, Houston, USA; cat. 31H-G1b). The HRP is subsequently quantified by
- 20 means of a stain reaction with 3,3',5,5'-tetramethylbenzidine (TMB) as substrate, and the stain intensity is compared with a calibration curve which is constructed with pure human apoCI (Labconsult, Brussels, Belgium; cat. A50366H).
- 25 Those skilled in the art, of course, know many variants of this apoCI determination. Thus, other assay techniques than ELISA can be used, other detection systems (enzymes and substrates), etc.

EXAMPLES

The finding that apoCI (and peptides derived therefrom) play a protective role in Gram-negative (and

5 Gram-positive) sepsis is supported by a number of experiments. To that purpose, use is made of:

- a. blood of septic patients;
- b. mice that do not synthesize apoCI (*apoc1*^{-/-} mice);
- c. transgenic mice that overexpress human apoCI (*APOC1*
- 10 mice);
- d. apoCI which has been purified from human blood.

The findings are described in the Examples given hereinbelow.

15 Example 1: Predictive value of the apoCI level for the chance of survival in sepsis

We have measured the apoCI levels in the blood plasma of 17 septic patients for four weeks after admission to the clinic. For that purpose, we used the human apoCI-

20 specific sandwich ELISA as described above under F.

The results are shown in Figure 1.

Of the 17 septic patients who were admitted to the clinic with severe sepsis, blood samples were taken over a period of 28 days. In the blood plasma, the contents of

25 apoCI, triglycerides (TG) and total cholesterol (TC) were measured.

In Figure 1A the apoCI content is plotted against time. In Figure 1B the apoCI content has been corrected for the triglyceride content as a measure for the amount of

30 triglyceride-rich lipoproteins, and in Fig. 1C the apoCI content has been corrected for both triglycerides and cholesterol as a measure for the total amount of circulating lipoproteins. A distinction was made between the patients who survived the sepsis (closed symbols; n=9

35 ± SEM) and the patients who died from the sepsis within 30

days (open symbols; $n=8 \pm \text{SEM}$). The dotted lines indicate the values as found in healthy individuals.

All patients who were included in the investigation exhibited a strongly lowered apoCI level in the blood (ca. 1.6 mg/dL). The patients who died from the consequences of sepsis within four weeks, exhibited a lower apoCI level (ca. 1.3 mg/dL) at the time of admission than did the patients who survived the sepsis (ca. 1.9 mg/dL). While the apoCI level virtually normalized within four weeks in patients who survived the sepsis (ca. 5 mg/dL), the apoCI level did not exhibit any rise in the patients who eventually died from sepsis.

These data show that the plasma level of apoCI has a predictive value for the chance of survival in sepsis. Also, they underline our hypothesis that apoCI falls short in patients who die from sepsis, and which can therefore have a therapeutic use.

Example 2: Purified apoCI binds strongly to LPS

In this Example, it is demonstrated that purified human apoCI binds strongly to LPS and that this binding is resistant to electrophoretic forces (agarose gel electrophoresis). It was found this involved the loss of the naturally occurring micellary structure of LPS.

The results are shown in Figures 2, 3 and 4.

LPS Re595 (*Salmonella minnesota*) was radioactively labeled with ^{125}I as described (Rensen, 1997) and incubated (30 min at 37°C) with increasing amounts of human apoCI which has been isolated from human blood (Tournier, 1984). Next, the incubation volumes were transferred to an agarose gel (position R_f 0), after which, for 2 hours, an electrophoretic field was applied (so-called agarose gel electrophoresis). After electrophoresis, the ^{125}I -LPS was visualized by means of autoradiography. Through its negative charge, LPS tends to migrate to the positive pole

(anode), which, however, is prevented in that the LPS forms large micellary structures that cannot pass the relatively small pores formed by the agarose matrix. However, addition of a small amount of apoCI (only 0.5 molecules of apoCI
5 with respect to 1 molecule of LPS) already led to complete migration of the LPS to the positive pole, which indicates that apoCI binds to LPS thereby forming relatively small apoCI/LPS complexes (Figure 2A):

Also, the effect of increasing amounts of LPS on the
10 migration of a fixed amount of apoCI was determined. For that purpose, the apoCI was visualized in the gel after electrophoresis by means of immunoblotting with a horseradish peroxidase-conjugated antibody directed against human apoCI (Academy Bio-Medical Company, Houston, USA;
15 cat. 31H-G1b). The binding of LPS to apoCI led to accelerated migration of apoCI to the positive pole, which indicates that apoCI in effect enters into a physical interaction with LPS (Figure 2B). The affinity of the binding of LPS to apoCI proves to be so high that LPS
20 prevents demonstration of apoCI during immunoblotting of the agarose gel with the apoCI-specific antibody in a dose-dependent manner (Figure 2B).

A particular amount of human apoCI (0.75 ng/mL) was incubated with LPS in increasing molar ratios, after which
25 the apoCI concentration was determined with the aid of the human apoCI-specific sandwich ELISA as described above under F. The LPS-induced inhibition of the stain reaction from which the apoCI concentration is read, was determined as a percentage of the maximum staining in the absence of
30 LPS ($n=2 \pm \text{SEM}$) (Figure 3). Again, the binding of LPS to apoCI proved to prevent the reaction of apoCI with the apoCI-specific antibody in a dose-dependent manner. Addition of a 200-fold molar excess of LPS to apoCI led to more than 40% inhibition of the staining reaction, that is,
35 a nearly twofold underestimation of the apoCI

concentration. Although apoCIII has a size (79 amino acids) and structure (two amphipathic helices) equal to apoCI, LPS had no effect on the quantification of concentrations of apoCIII with the aid of a comparable apoCIII-specific sandwich ELISA (Figure 3).

Finally, the effect of apoCI on the monomerization rate of LPS was studied. Fluorescent (fluorescein isothiocyanate; FITC)-labeled LPS hardly exhibits fluorescence, because the micellar structure of LPS leads to the fluorescing FITC molecules being too closely packed together, giving rise to the phenomenon of fluorescence quenching. FITC-LPS Re595 (100 ng) was incubated with pure human apoCI (in molar ratios 1:1, 1:5, 1:10 and 1:20), after which the occurrence of fluorescence was followed in time with the aid of a fluorimeter. ApoCI proved to cause the increase in FITC fluorescence to increase strongly in a dose-dependent manner, which indicates that apoCI is capable of monomerizing the LPS (i.e. to liberate it from the micellar form) (Figure 4A). By contrast, the apolipoprotein CIII (apoCIII), having a comparable size and structure, proved hardly effective (Figure 4B).

Example 3: Plasma apoCI binds to LPS

We have determined the effect of the apoCI level in murine plasma on monomerization of FITC-LPS as described in Example 2. For that purpose, FITC-LPS was incubated with plasma that had been isolated from wild-type mice, genetically modified mice which are deficient for apoCI (*apoc1*^{-/-}), or genetically modified mice which express the human apoCI hemizygote (*APOC1*^{+/-}) or homozygote (*APOC1*^{+/+}).

The results are shown in Figure 5.

FITC-LPS Re595 (100 ng) was incubated with plasma (0.125%) isolated from the blood of the various mouse types, after which the occurrence of fluorescence was followed in time with the aid of a fluorimeter. The

expression of human apoCI in mouse plasma led to an apoCI-dose dependent increase of the FITC-LPS monomerization, which was 32% at a maximum. By contrast, the monomerization of FITC-LPS proved to be reduced by 60% owing to the
5 absence of mouse-apoCI ($n=4 \pm \text{SEM}$). This indicates not only that mouse-apoCI, like human apoCI, is capable of monomerizing LPS, but especially also that the endogenous presence of apoCI contributes for a considerable part to the total LPS-disaggregated capacity of mouse plasma.

10

Example 4: ApoCI extends the residence time of LPS in the blood by preventing the uptake of LPS by the liver

We have administered LPS intravenously to wild-type mice in the absence or presence of purified apoCI. Addition
15 of apoCI proved to extend the residence time of LPS in the blood strongly through increased interaction with HDL.

The results are shown in Figure 6.

Wild-type (C57Bl/6) mice were anesthetized and, via the lower vena cava, injected intravenously with
20 radioactively (^{125}I) labeled LPS Re595 (10 $\mu\text{g/kg}$) without, and with, respectively, apoCI (molar ratio LPS:apoCI = 1:0.2, 1:0.5, 1:1 and 1:16) and bovine serum albumin (molar ratio LPS:albumin = 1:16). Blood samples and bits of liver tissue were taken at $t = 2, 5, 10, 20$ and 30 minutes after
25 injection. After this, the mice were sacrificed. Next, the amounts of LPS in the blood sera and liver samples were quantified on the basis of the amount of radioactive radiation. Also, a serum sample was taken 30 minutes after injection of LPS:apoCI (1:1) and subjected to agarose gel
30 electrophoresis to study the distribution of the ^{125}I -LPS over the various lipoproteins. Lipoprotein lipids were visualized with the aid of Sudan Black, and ^{125}I -LPS by means of autoradiography. In Figures 6A and 6B the amounts of ^{125}I -LPS in the liver and the blood serum, respectively,
35 are plotted as a percentage of the injected dose against

time ($n=3 \pm \text{SEM}$). Figure 6C shows the distribution of ^{125}I -LPS over the lipoproteins. The presence of apoCI was found to inhibit the decrease of LPS from the blood strongly and dose-dependently. Already at a molar ratio of 1:1, the uptake of LPS by the liver was reduced 8-fold. The strong binding of apoCI to LPS (see Example 2) therefore proves to be relevant in the blood too. This led to an extended residence time of LPS in the blood through a strongly increased apoCI-mediated binding to HDL (Figure 6C), thereby improving the interaction with *inter alia* white blood cells and eventually leading to a strongly stimulated inflammation reaction (see Example 5).

Example 5: ApoCI stimulates the LPS-induced inflammatory response in mice

We have administered LPS intravenously to wild-type mice in the absence or presence of purified apoCI. Addition of apoCI proved to increase the LPS-induced inflammatory response strongly.

The results are shown in Figure 7.

Wild-type (C57Bl/6) mice were injected, by way of the tail vein, with LPS Re595 (25 $\mu\text{g/kg}$) without, and with, apoCI (molar ratio LPS:apoCI = 1:5). Blood samples were taken at $t = 0, 30, 60, 90, 120$ and 180 minutes after injection. In the samples, subsequently, the amount of $\text{TNF}\alpha$ was quantified with the aid of a mouse- $\text{TNF}\alpha$ specific ELISA according to the accompanying instructions (Immunosource, Halle, Belgium, cat. no. BMS607MST), and the amounts of $\text{TNF}\alpha$ in the blood plasma were subsequently plotted against time. Addition of apoCI to LPS proved to lead to a fourfold increase of the LPS-induced $\text{TNF}\alpha$ level (10.8 ± 4.5 versus 2.8 ± 1.7 ng/mL), while an equal amount of apoCI without LPS has no $\text{TNF}\alpha$ -inducing effect ($n=6 \pm \text{SEM}$).

Example 6: ApoCI stimulates the LPS-induced inflammatory response in humans

We have measured the correlation between plasma apoCI levels in patients who did or did not develop endotoxemia during a heart operation with cardiopulmonary bypass, and the TNF α level during the operation. The apoCI level proved to be strongly positively correlated to the TNF α response in case of a developed endotoxemia.

The results are shown in Figure 8.

During a cardiopulmonary bypass, temporarily a low oxygen pressure arises in the blood which is accompanied by an endotoxemia through leakage of LPS from the intestines to the blood. We investigated the correlation between the preoperative plasma apoCI level and the plasma TNF α level in 160 patients who had a heart operation involving a cardiopulmonary bypass. Plasma samples were taken before the operation for the purpose of measurements of lipid parameters (apoCI, apoCIII, apoE, triglycerides and total cholesterol). Also, plasma samples were taken after inducing anesthesia (time 1), after removal of the aorta clamp (time 2) and half an hour after termination of the extracorporeal perfusion (time 3). At times 1, 2 and 3 the TNF α level was determined, with a distinction being made between patients who did (LPS >5 pg/mL) or did not (LPS <5 pg/mL) develop endotoxemia at both time 2 and 3.

R = Spearman's rank correlation coefficient.

* = statistically significant (P<0.05).

A strongly significant relation was found to exist between the apoCI level and the TNF α level in patients who during a heart operation with cardiopulmonary bypass developed endotoxemia, but not in patients who did not develop endotoxemia. Such a correlation is not demonstrated for other apolipoproteins (apoCIII, apoE) or neutral lipids (triglycerides as a measure for the amount of triglyceride-rich lipoproteins, and total cholesterol as a measure for

the total amount of circulating lipoproteins), which indicates that the effect is apoCI-specific.

REFERENCES

- Angus, D.C., Linde-Zwirble, W.T., Lidicker, J., Clermont, G., Carcillo, J., Pinsky, M.R. (2001)
5 Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit. Care Med. 29, 1303-1310.
- Barlage, S., Frohlich, D., Bottcher, A. (2001) ApoE-containing high density lipoproteins and phospholipid
10 transfer protein activity increase in patients with a systemic inflammatory response. J. Lipid Res. 42, 281-290.
- Bozza, M., Satoskar, A.R., Lin G., Lu, B., Humbles, A.A., Gerard, C., David, J.R. (1999) Targeted disruption
15 of migration inhibitory factor gene reveals its critical role in sepsis. J. Exp. Med. 189, 341-346.
- Buclin, T., Cosma Rochat, M., Burckhardt, P., Azria, M., Attinger, M. (2002) Bioavailability and biological
20 efficacy of a new oral formulation of salmon calcitonin in healthy volunteers. J. Bone Miner. Res. 17:1478-1485.
- Calandra, T., Echtenacher, B., Roy, D.L., Pugin, J., Metz, C.N., Hultner, L., Heumann, D., Mannel, D., Bucala, R., Glauser, M.P. (2000) Protection from septic
25 shock by neutralization of macrophage migration inhibitory factor. Nat. Med. 6, 164-170.
- Clark, M.A., Plank, L.D., Connolly, A.B. et al. (1998) Effect of a chimeric antibody to tumor necrosis factor- α on cytokine and physiologic responses in patients with
30 severe sepsis: a randomized, clinical trial. Crit. Care Med. 26, 1650-1659.
- Clark-Lewis, I., Aebbersold, R., Ziltener, H., Schrader, J.W., Hood, L.E., Kent, S.B. (1986) Automated chemical synthesis of a protein growth factor for hemopoietic cells, interleukin-3. Science 231, 134-139.

- Cohen, J., Glauser, M.P. (1991) Septic shock: treatment. Lancet 338, 736-739.
- Cohen, J. (1999) Adjunctive therapy in sepsis: a critical analysis of the clinical trial programme. Br. Med. Bull. 55, 212-225.
- Cronin, E., Cook, D.J., Carlet, J. et al. (1995) Corticosteroid treatment for sepsis: a critical appraisal and meta-analysis of the literature. Crit. Care. Med. 23, 1430-1439.
- Curry, M.D., McConathy, W.J., Fesmire, J.D., and Alaupovic, P. (1981) Quantitative determination of apolipoproteins C-I and C-II in human plasma by electroimmunoassays. Clin. Chem. 27, 543-548.
- Fisher, C.J., Dhainaut, J.F., Opal, S.M. (1994) Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome: results from a randomized double-blind, placebo-controlled trial. JAMA 271, 1836-1843.
- Fisher, C.J., Agosti, J.M. Opal, S.M. et al. (1996) Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. The Soluble TNF Receptor sepsis study Group. N. Engl. J. Med. 334, 1697-1702.
- Heumann, D., Roger, T. (2002) Initial responses to endotoxins and Gram-negative bacteria. Clin. Chim. Acta 323, 59-72.
- Hoess, A., Watson, S., Siber, G.R., Liddington, R. (1993) Crystal structure of an endotoxin-neutralizing protein from the horseshoe crab, Limulus anti-LPS factor, at 1.5Å resolution. EMBO J. 12, 3351-3356.
- Jackson, R.L., Holdsworth, G. (1986) Isolation and properties of human apolipoproteins C-I, C-II, and C-III. Methods Enzymol. 128, 288-297.
- Jong, M.C., Dahlmans, V.E.H., Van Gorp, P.J.J. et al. (1996) In the absence of the low density receptor, human apolipoprotein C1 overexpression in transgenic mice

inhibits the hepatic uptake of very low density lipoproteins via a receptor-associated protein-sensitive pathway. J. Clin. Invest. 98, 2259-2267.

- Jong, M.C., Gijbels, M.J.J., Dahlmans, V.E.H. et al.
5 (1998) Hyperlipidemia and cutaneous abnormalities in transgenic mice overexpressing apolipoprotein C1. J. Clin. Invest. 101, 145-152.
- Jong, M.C., Hofker, M.H., Havekes, L.M. (1999) Role of
10 apoCs in lipoprotein metabolism: functional differences between apoC1, apoC2, and apoC3. Artheroscler. Thromb. Vasc. Biol. 19, 472-484.
- Koebernick, H. et al. (2002) Macrophage migration
inhibitory factor (MIF) plays a pivotal role in immunity
15 against *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA 99, 13681-13686.
- Kiechl, S. Lorenz, E., Reindl, M., Wiedermann, C.J.,
Oberhollenzer, F., Bonora, E., Willeit, J., Schwartz,
D.A. (2002) Toll-like receptor 4 polymorphisms and
atherogenesis. N. Engl. J. Med. 347, 185-192.
- 20 - Kypreos, K.E., Willems van Dijk, K., Van der Zee, A.,
Havekes, L.M., Zannis, V.I. (2001) Domains of
apolipoprotein E contributing to triglyceride and
cholesterol homeostasis in vivo. J. Biol. Chem. 276,
19778-19786.
- 25 - Larrick, J.W., Hirata, M., Zheng, H. et al. (1994) A
novel granulocyte-derived peptide with
lipopolysaccharide-neutralizing activity. J. Immunol.
152, 231-240.
- Opal, S.M., Fisher, C.J., Dhainaut, J.F. et al. (1997)
30 Confirmatory interleukin-1 receptor antagonist trial in
severe sepsis: a phase III, randomized, double-blind,
placebo-controlled, multicenter trial. The Interleukin-1
Receptor Antagonist Sepsis Investigator Group. Crit.
Care Med. 26, 1115-1124.

- Rackow, E.C., Astiz, M.E. (1991) Pathophysiology and treatment of septic shock. JAMA 266, 548-554.
- Rensen, P.C.N., Van Oosten, M., Van de Bilt, E. et al. (1997) Human apolipoprotein E protects redirects
5 lipopolysaccharide from Kupffer cells to liver parenchymal cells in rats in vivo. J. Clin. Invest. 99, 2438-2445.
- Satoskar, A.R., Bozza, M., Rodriguez Sosa, M., Lin, G., David, J.R. (2001) Migration-inhibitory factor gene-
10 deficient mice are susceptible to cutaneous *Leishmania* major infection. Infect. Immun. 69, 906-911.
- Tournier, J.F., Bayard, F., Tauber, J.P. (1984) Rapid purification and activity of apolipoprotein C_I on the proliferation of bovine vascular endothelial cells in
15 vitro. Biochim. Biophys. Acta 804, 216-220.
- Van der Poll, T., Braxton, C.C., Coyle, S.M. et al. (1995) Effect of hypertriglyceridemia on endotoxin responsiveness in humans. Infect. Immun. 63, 3396-3400.
- Wheeler, A.P., Bernard, G.R. (1999) Treating patients
20 with severe sepsis. N. Engl. J. Med. 340, 207-214.

CLAIMS

1. Use of a peptide which binds to a lipopolysaccharide (LPS) or lipoteichoic acid (LTA), for manufacturing a pharmaceutical composition for treating sepsis or septic shock, wherein the peptide comprises the amino acid
5 sequence of apolipoprotein CI (apoCI) or a part thereof that comprises at least the amino acids of the C-terminal helix of apoCI.
2. Use according to claim 1, wherein the peptide comprises the amino acid sequence of human apoCI or a part
10 thereof that comprises at least the amino acids of the C-terminal helix of human apoCI.
3. Use according to claim 2, wherein the peptide is human apoCI or a fragment thereof that comprises at least the amino acids MREWFSETFQKVKEKLK.
- 15 4. Use according to claim 3, wherein the peptide is human apoCI having the amino acid sequence
TPDVSSALDKLKEFGNTLEDKARELIS RIKQSELSAKMREWFSETFQKVKEKLKIDS.
5. Use of a peptide which binds to lipopolysaccharide (LPS) according to any one of claims 1-4, wherein a
20 pharmaceutical composition is manufactured for preventing or treating a sepsis or septic shock caused by Gram-negative bacteria in mammals, in particular humans or a domestic animal, such as horse, cow, dog and cat.
6. Use of a peptide which binds to lipoteichoic acid (LTA) according to any one of claims 1-4, wherein a
25 pharmaceutical composition is manufactured for preventing or treating a sepsis or septic shock caused by Gram-positive bacteria in mammals, in particular humans or a domestic animal, such as horse, cow, dog and cat.
- 30 7. A pharmaceutical composition for preventing or treating sepsis or septic shock, which composition

comprises a peptide as defined in one or more of the claims 1-4 as well as a pharmaceutically acceptable carrier.

8. A method for preventively treating a mammal, in particular human individual, which, for instance as a result of a surgical intervention or a weakened immune system, is at increased risk of developing sepsis, wherein to the mammal an active amount is administered of a peptide as defined in one or more of claims 1-4.

9. A method for treating a mammal, in particular human individual, which suffers from sepsis or septic shock, wherein to the mammal an active amount is administered of a peptide as defined in one or more of claims 1-4.

10. A method for determining the severity of a septic condition and making a prognosis for the further course of the sepsis or septic shock in a mammal, in particular human individual, which suffers from sepsis or septic shock, wherein the apoCI content is determined in a blood sample of the mammal.

11. A method for monitoring a treatment of sepsis or septic shock in a mammal, in particular human individual, which is being treated for sepsis or septic shock, wherein the apoCI content is determined in a blood sample of the mammal.

Figure 1
Predictive value of the apoCl content in blood plasma for
the chance of survival of sepsis

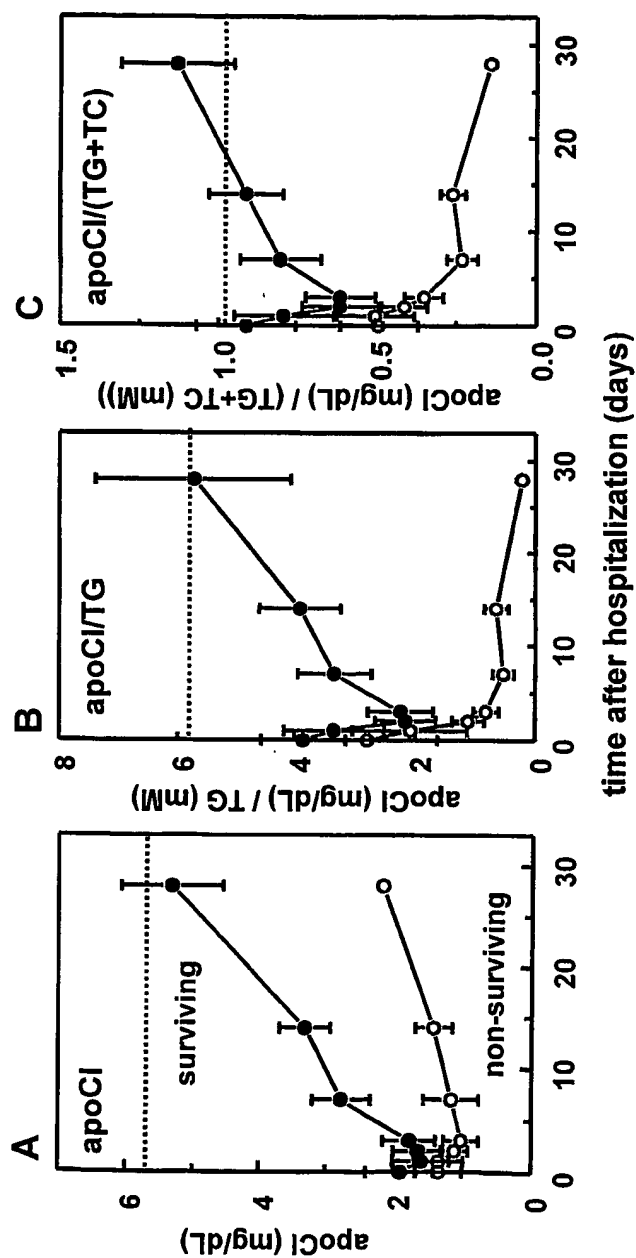


Figure 2

ApoCI shows strong binding to LPS, which is resistant to an electrophoretic field

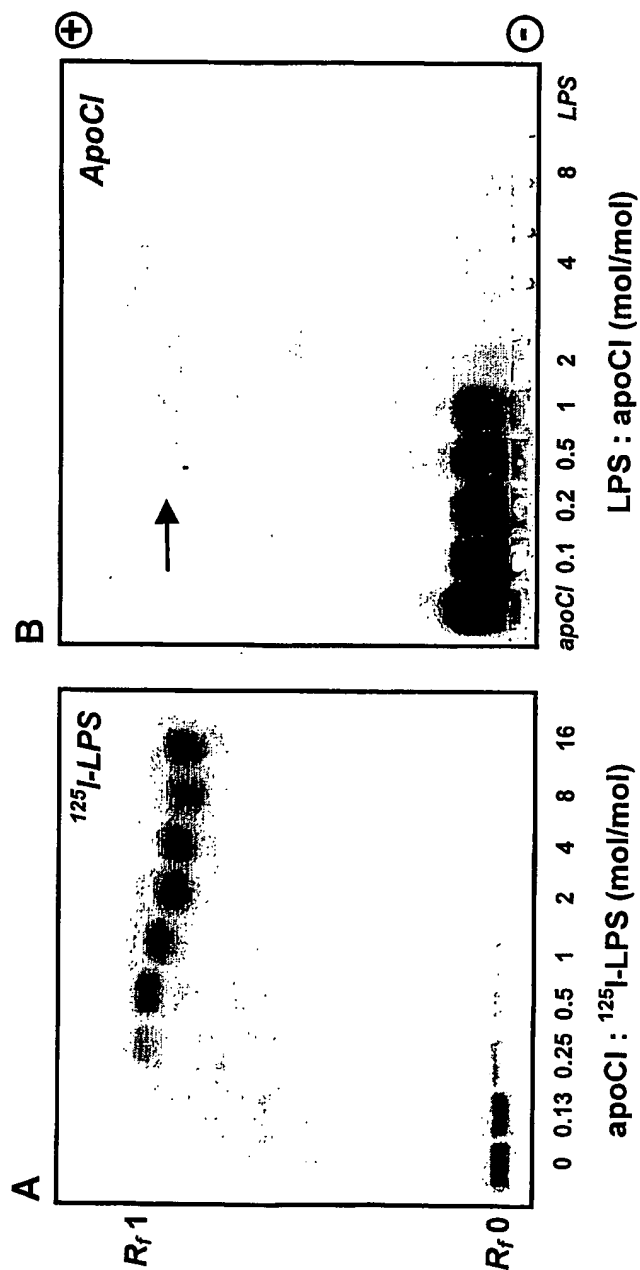


Figure 3

The interaction of apoCI with LPS inhibits the interaction of an apoCI-specific antibody with apoCI

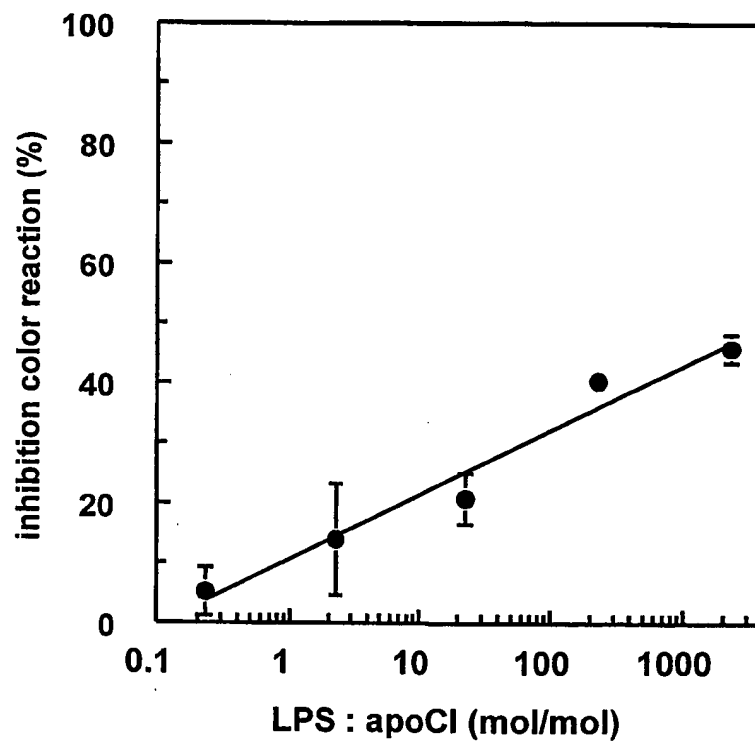


Figure 4

The binding of apoCI to LPS leads to monomerization of apoCI micells

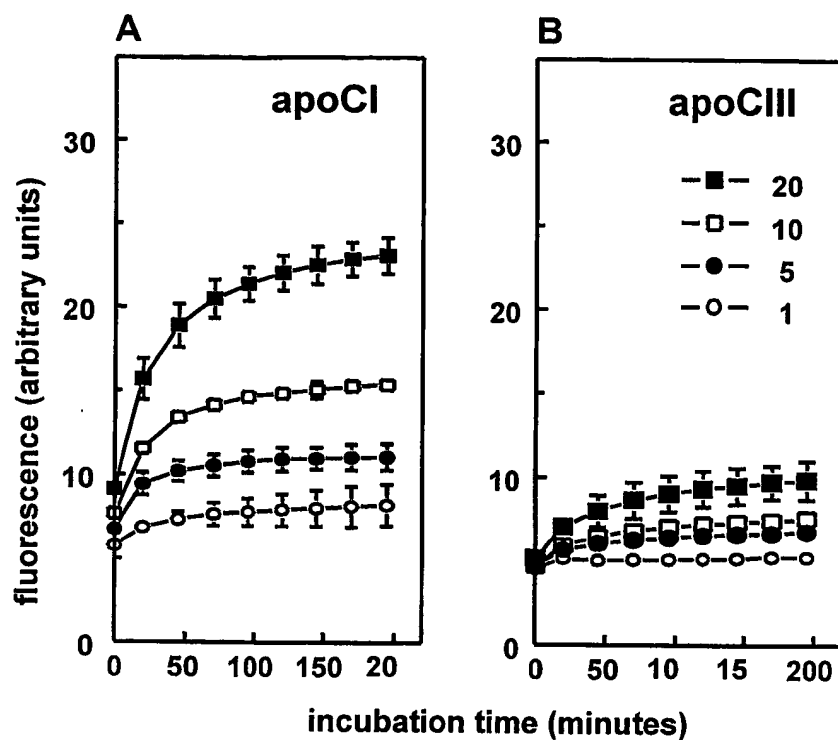


Figure 5

The rate and degree of LPS monomerization by mice plasma is determined by the apoC1 concentration

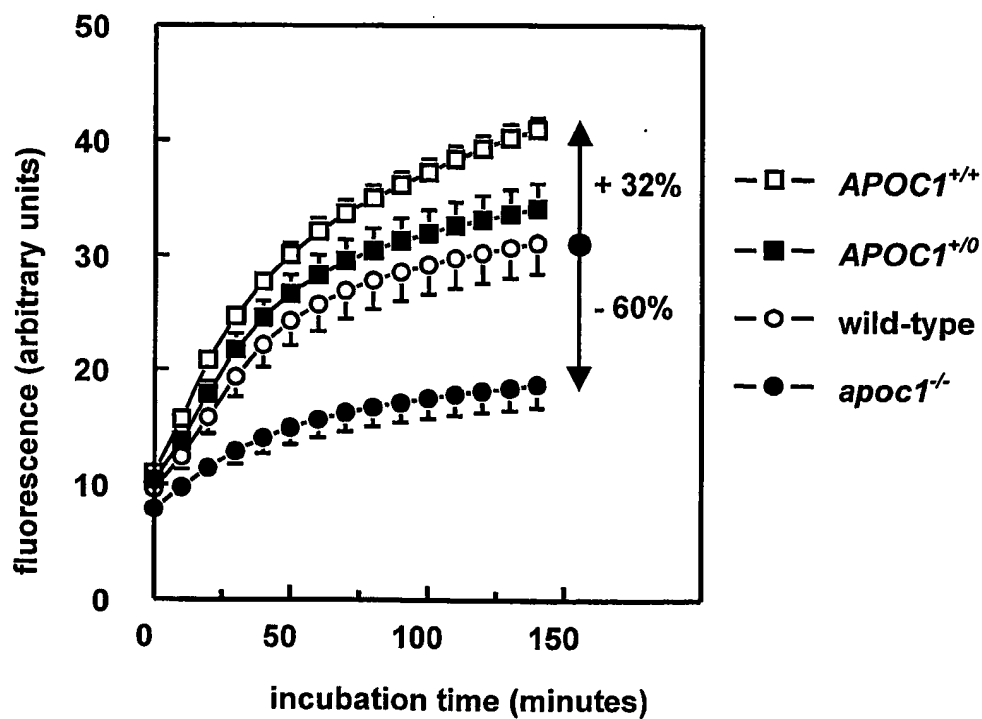


Figure 6

Binding of LPS to human apoC1 leads to a strongly reduced interaction with the liver, while the residence time of LPS in the blood increases considerably

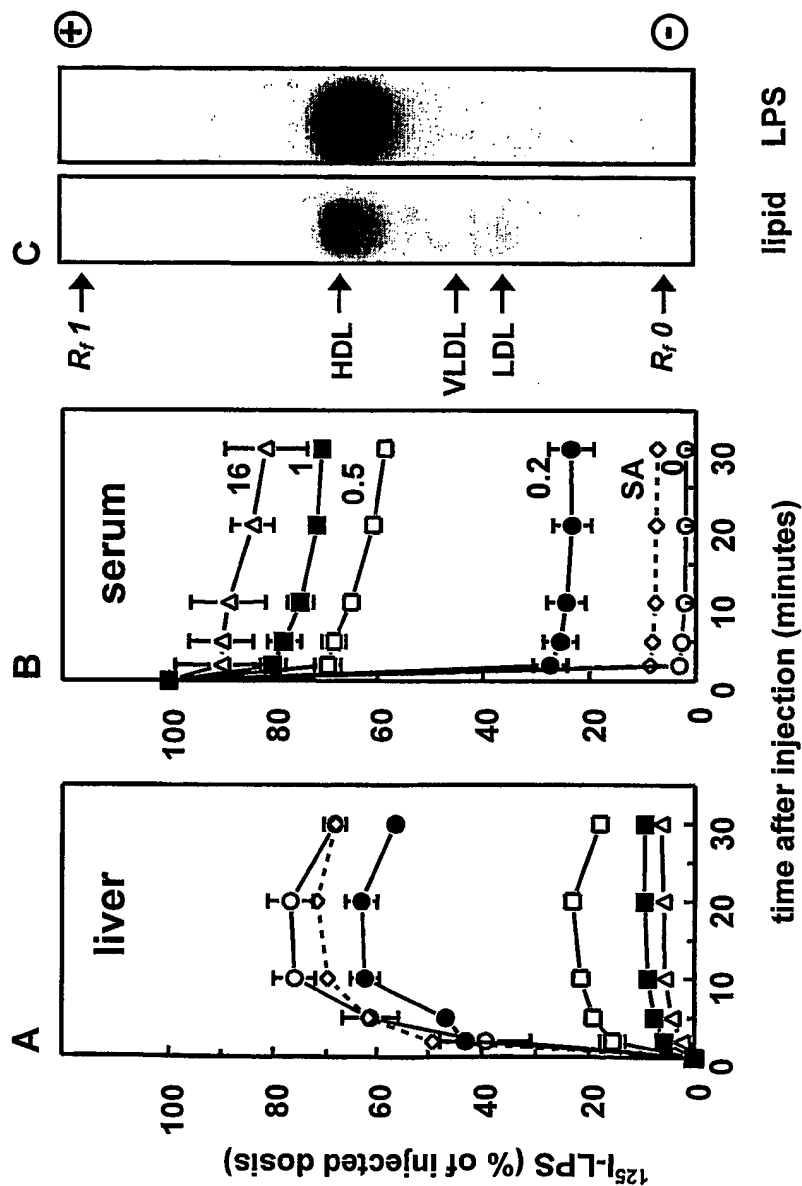


Figure 7

Binding of LPS to human apoCI leads to an intensified LPS-induced inflammatory response

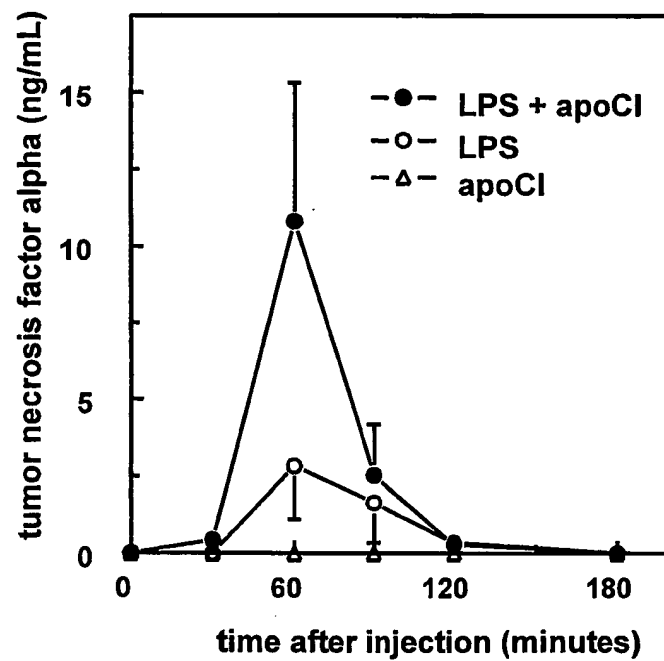


Figure 8

Plasma apoCI levels are positively correlated with TNF α levels in patients developing endotoxemia during a heart operation with cardiopulmonary bypass

	<i>time point 1</i>		<i>time point 2</i>		<i>time point 3</i>	
	R	P	R	P	R	P
ApoCI						
all patients	0.150	0.128	0.290	0.004*	0.289	0.003*
LPS <5 pg/mL	0.165	0.464	0.300	0.176	0.178	0.440
LPS >5 pg/mL	0.141	0.216	0.293	0.010*	0.317*	0.004*
ApoCIII						
all patients	0.090	0.364	-0.145	0.152	0.005	0.960
LPS <5 pg/mL	-0.044	0.846	-0.020	0.931	0.070	0.764
LPS >5 pg/mL	0.121	0.289	-0.159	0.168	0.008	0.947

INTERNATIONAL SEARCH REPORT

Internat. Application No
P 03/00475A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/17 A61M1/36 G01N33/68 A61P31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61M C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, PAJ, WPI Data, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 747 455 A (NOVITSKY THOMAS J ET AL) 5 May 1998 (1998-05-05) column 2, line 55 -column 3, line 20; claims; figures 15,16; examples ---	1-11
A	J.W. LARRICK ET AL.: "A novel granulocyte-derived peptide with lipopolysaccharide-neutralizing activity." JOURNAL OF IMMUNOLOGY, vol. 152, 1994, pages 231-240, XP002236820 BALTIMORE, US cited in the application page 236, right-hand column, line 5 -page 238, right-hand column, last line; figure 1; table III --- -/--	1-11



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

14 October 2003

Date of mailing of the international search report

23/10/2003

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Ryckebosch, A

INTERNATIONAL SEARCH REPORT

Internati... application No
P0... 03/00475

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 17740 A (DASSEUX JEAN LOUIS) 15 April 1999 (1999-04-15) page 6, line 23 - line 37; claims 1-5, 10-15, 31-34 page 7, line 34 - page 8, line 2 page 18, line 1 - line 13 ---	1-11
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; February 2001 (2001-02) BARLAGE STEFAN ET AL: "ApoE-containing high density lipoproteins and phospholipid transfer protein activity increase in patients with a systemic inflammatory response." Database accession no. PREV200100192387 XP002236821 abstract & JOURNAL OF LIPID RESEARCH, vol. 42, no. 2, February 2001 (2001-02), pages 281-290, ISSN: 0022-2275 cited in the application -----	1-11

INTERNATIONAL SEARCH REPORT

International application No.
NL 03/00475

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 8 and 9 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP 03/00475

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5747455	A	05-05-1998	US 5594113 A	14-01-1997
			US 6222021 B1	24-04-2001
			US 5627266 A	06-05-1997
			US 5614369 A	25-03-1997
			US 6384200 B1	07-05-2002
			AU 2006892 A	30-12-1992
			DE 69232307 D1	31-01-2002
			DE 69232307 T2	18-07-2002
			EP 0586573 A1	16-03-1994
			WO 9220715 A1	26-11-1992
			AT 207205 T	15-11-2001
			AU 3772289 A	12-01-1990
			CA 1338836 C	07-01-1997
			DE 68925275 D1	08-02-1996
			DE 68925275 T2	22-08-1996
			DE 68929334 D1	22-11-2001
			EP 0379549 A1	01-08-1990
			EP 0687911 A2	20-12-1995
			JP 2774343 B2	09-07-1998
			JP 3501733 T	18-04-1991
			WO 8912644 A1	28-12-1989
WO 9917740	A	15-04-1999	US 6287590 B1	11-09-2001
			AU 749344 B2	27-06-2002
			AU 9671598 A	27-04-1999
			CA 2305704 A1	15-04-1999
			CN 1280490 T	17-01-2001
			EP 1019025 A1	19-07-2000
			HU 0003930 A2	28-05-2001
			JP 2001522781 T	20-11-2001
			NO 20001683 A	10-05-2000
			NZ 503721 A	31-08-2001
			PL 339654 A1	02-01-2001
			WO 9917740 A1	15-04-1999
			US 6455088 B1	24-09-2002